The effect of inspired oxygen concentration on oxidative stress biomarkers in dogs under inhalation anesthesia

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Abstract

This study investigated oxidative stress biomarkers at 3 different oxygen concentrations in dogs under general anesthesia to determine whether high-concentration oxygen increases oxidative stress. Six healthy beagles were randomly assigned to receive 3 anesthesia protocols (inhalation of 40%, 60%, and 100% oxygen) during 3 hours of general anesthesia with sevoflurane, with at least one week in between each protocol. For each experiment, blood samples were collected at 0, 3, 6, and 24 hours after inhalation of oxygen. Derivatives of reactive oxygen metabolites, biochemical antioxidant potential, superoxide dismutase, and 8-hydroxydeoxyguanosine in the blood did not significantly differ among the 3 groups at any time point. This study is the first comparing high concentrations of oxygen with low concentrations of oxygen for anesthesia in dogs. According to our findings, 100% oxygen may not alter the oxidative stress level in dogs during general anesthesia with sevoflurane for 3 hours.

Résumé

La présente étude a examiné les biomarqueurs oxydatifs de stress à trois concentrations différentes en oxygène chez des chiens sous anesthésie générale afin de déterminer si des concentrations élevées en oxygène augmentent le stress oxydatif. Six chiens beagles en santé ont été assignés de manière aléatoire pour recevoir trois protocoles d'anesthésie (inhalation de 40 %, 60 % et 100 % d'oxygène) pendant 3 heures d'anesthésie générale avec du sévoflurane, avec au moins une semaine entre chaque protocole. Pour chaque essai, des échantillons sanguins furent prélevés à 0, 3, 6 et 24 heures après l'inhalation d'oxygène. Des dérivés de métabolites oxygène réactif, le potentiel anti-oxydant biochimique, la superoxyde dismutase et le 8-hydroxydéoxyguanosine dans le sang n'ont pas différé significativement parmi les trois groupes à n'importe quel moment. Cette étude est la première à comparer des concentrations élevées en oxygène avec des concentrations faibles en oxygène lors d'anesthésie chez des chiens. Selon nos trouvailles, 100 % d'oxygène ne modifierait pas le niveau de stress oxydatif chez les chiens durant une anesthésie générale avec du sévoflurane pendant 3 heures.

(Traduit par Docteur Serge Messier)

Introduction

Oxygen is necessary for maintaining life in humans and animals, as it is used during cellular inspiration and energy production. Moreover, oxygen is a therapeutic agent commonly used in medical treatment (1–4). It is frequently applied in clinical veterinary practice (5) and is required in inhalation anesthesia to deliver anesthesia gas and maintain breathing (6).

The usefulness of oxygen therapy has been proven in many studies; in particular, high-concentration oxygen (> 80%) can be used to protect against infection and has been shown to aid in wound healing in colorectal surgery patients (7). However, oxygen is often used incorrectly and the risks of excessive oxygenation are sometimes disregarded. It is widely suspected that excessive oxygenation increases oxidative stress, which is related to atherosclerosis, cataracts, retinopathy, myocardial infarction, hypertension, diabetes, and renal failure (2–4,8).

Many reports have indicated that 100% oxygen is unnecessary for anesthesia of human infants (8,9). Currently, 100% oxygen during anesthesia induction is preferred for high-risk patients. However, this high concentration may cause a variety of complications (7). A study in 2010 showed that the mortality rate increased by 5% when high-concentration oxygen was administered to chronic obstructive pulmonary disease patients (1).

Importantly, 100% and 60% oxygen have been shown to increase dogs' arterial blood partial pressure of oxygen to 535 mmHg and 374 mmHg, respectively, during a 3-hour general anesthesia procedure (10). However, there is a lack of studies regarding the usefulness of 100% oxygen during general anesthesia in dogs.

The objectives of the present study were to investigate whether 100% oxygen is excessive in veterinary medicine applications and to determine an optimal level of oxygen for lowering oxygen toxicity. We hypothesized that the use of 100% oxygen during general

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Received April 29, 2019. Accepted June 16, 2019.

anesthesia would increase oxidative stress in dogs relative to lower concentrations.

Materials and methods

Dogs

Six healthy beagles (4 males and 2 females; mean weight = 16.18 kg) were used in this study. This study was performed in accordance with guidelines approved by the Experiment Animal Use Committee of Iwate University (approval number A201560).

Induction and general anesthesia

Six dogs received 3 anesthesia protocols during 3 h of general anesthesia with sevoflurane (Sevofrane; Maruishi Pharmaceutical, Osaka, Japan). The dogs were anesthetized 3 times (inhalation of 40%, 60%, and 100% O_2), with at least 1 wk between each occasion. The order of the anesthesia protocols was randomly assigned. Regulated respiration was performed with a ventilator (Isepo; Acoma Medical, Tokyo, Japan).

An intravenous (IV) catheter was inserted into the cephalic vein of each dog. Then, the dog received atropine 0.04 mg/kg body weight (BW), subcutaneously (atropine sulfate hydrate 0.5 mg; Fuso Pharmaceutical Industries, Osaka, Japan), midazolam 0.3 mg/kg BW, IV (Dormicum; Astellas Pharma, Tokyo, Japan), and butorphanol 0.2 mg/kg BW, IV (Vetorphal; Meiji Seika Pharma, Tokyo, Japan), 45 min before the start of the experiment. Anesthesia was then induced with propofol 7 mg/kg BW, IV (Animal Propofol Mylan 1%; Intervet, Tokyo, Japan). An endotracheal tube (wire-reinforced endotracheal tube; Fuji Systems, Tokyo, Japan) was inserted when the dog was sufficiently anesthetized. For maintenance of anesthesia, propofol was continuously administered. Each dog then was administered inhaled sevoflurane (2% to 3%) with the predetermined oxygen concentration for 3 h. The initiation of oxygen inhalation was designated as 0 h. The flow rate of O_2 or O_2 and air was set at 3 L/min. During anesthesia, lactated Ringer's solution 3 mL/kg/BW per hour (Solulact; Terumo, Tokyo, Japan) was administered to the cephalic vein through the venous catheter. Vital signs [heart rate (HR), respiratory rate (RR), electrocardiogram, systolic aortic pressure (SAP), diastolic aortic pressure (DAP), end-tidal carbon dioxide ($EtCO_2$), and peripheral capillary oxygen saturation (SpO_2)] were monitored during general anesthesia.

Blood sample collection

Blood samples were collected before induction (baseline) and 3, 6, and 24 h after inhalation of oxygen. During each sample collection, 7 mL of blood was collected from the femoral or cephalic vein. Blood samples were centrifuged at 1500 × *g* for 15 min at 4°C and plasma was collected and stored at −80°C until required for analysis.

Assessment of plasma d-ROMs and BAP

The levels of derivatives of reactive oxygen metabolites (d-ROMs) and biochemical antioxidant potential (BAP) were measured with a device for determining free radical and antioxidant potential (Free Radical Analytical System 4; H&D, Parma, Italy). The d-ROMs were used to determine the level of oxidative stress by measuring the amount of organic hydroperoxide converted into radicals that oxidize N,N-diethyl-p-phenylenediamine. The BAP was used to determine the biological antioxidant capacity and measured according to the capacity of the plasma to reduce ferric iron to ferrous iron.

Assessment of plasma SOD and 8-OHdG concentrations

Plasma superoxide dismutase (SOD) and 8-hydroxydeoxyguanosine (8-OHdG) concentrations were measured with commercial ELISA test kits (SOD assay kit; Cayman Chemical, Michigan, USA; 8-OHdG assay kit; Cloud-Clone, Houston, Texas, USA). Absorption at a wavelength of 450 nm was measured using an ELISA reader. All samples were analyzed in duplicate.

Statistical analysis

All results are expressed as mean and standard error of the mean (SEM). Data were tested for normality using the Kolmogorov-Smirnov test prior to statistical comparisons. All data showed a non-normal distribution; thus, non-parametric statistical methods were used.

For each time point, the value for the experimental group was compared with that at baseline. Differences within groups were analyzed by the Mann-Whitney *U*-test followed by a Bonferroni correction, whereas differences among the 3 groups were analyzed by the Kruskal-Wallis test. Values of $P < 0.05$ were considered statistically significant. The Benjamini-Hochberg method was used to correct false discovery rates. All statistical analyses were performed with SPSS software (version 19.0; IBM, Armonk, New York, USA).

Results

Vital sign values (HR, RR, SAP, DAP, $EtCO_2$, and SpO_2) during anesthesia were within normal ranges. The means of vital signs are shown in Table I.

Plasma d-ROMs

Mean ± SEM plasma d-ROM concentrations are shown in Table II. There were no significant differences between baseline concentrations and those at each time point in any group, and there was no statistical difference among the 3 groups at any time point.

Plasma BAP

Mean ± SEM plasma BAP concentrations are shown in Table III. There were no significant differences between baseline concentrations and those at each time point in any group, and there was no statistical difference among the 3 groups at any time point.

Plasma SOD

Mean ± SEM plasma SOD concentrations are shown in Table IV. There were no significant differences between baseline concentrations and those at each time point in any group, and there was no statistical difference among the 3 groups at any time point.

Plasma 8-OHdG

Mean ± SEM plasma 8-OHdG concentrations are shown in Table V. There were no significant differences between baseline concentrations and those at each time point in any group, and there was no statistical difference among the 3 groups at any time point.

Table I. Means of vital signs in all dogs during 3 h of general anesthesia.

	Minutes							
	0	30	60	90	120	150	180	Total
HR (bpm[1])	120.9	104.8	101.6	99.1	97.7	94.2	97.7	100.8
RR (bpm[2])	11.6	11.8	13.2	13.2	12.4	12.6	10.6	12.5
SAP (mmHg)	105.8	102.3	105.7	111.6	115.1	120.8	119.2	111.5
DAP (mmHg)	67.7	64.3	65.3	67.9	71.1	74.2	73.1	68.9
$EtCO_2$ (%)	43.8	41.6	37.2	35.2	35.7	35.8	35.5	37.4
SpO_2 (%)	98.3	98.5	99.0	98.8	98.8	98.7	99.0	98.7

HR — heart rate; bpm[1] — beats/min; bpm[2] — breaths/min; RR — respiratory rate; SAP — systolic aortic pressure; DAP — diastolic aortic pressure; $EtCO_2$ — end-tidal carbon dioxide; SpO_2 — peripheral capillary oxygen saturation.

Table II. Mean ± SEM plasma d-ROM concentrations (U.CARR) in the 3 oxygen concentration groups at 0, 3, 6, and 24 h after initiation of sevoflurane. P_{hours} are the comparisons among the 3 groups at each time point and $P_{100\%}$, $P_{60\%}$, and $P_{40\%}$ are the comparisons between baseline and each time point in the 100%, 60%, and 40% groups, respectively.

Hours	P_{hours}	100% group	$P_{100\%}$	60% group	$P_{60\%}$	40% group	$P_{40\%}$
0	0.99	104.50 ± 5.98	—	103.50 ± 7.99	—	105.83 ± 7.89	—
3	0.64	91.50 ± 7.71	0.26	97.50 ± 7.93	0.38	102.83 ± 12.82	1.0
6	0.64	95.00 ± 4.77	0.38	104.50 ± 5.81	0.63	112.17 ± 12.65	1.0
24	0.48	99.17 ± 10.33	0.63	121.00 ± 15.30	0.52	115.50 ± 6.56	0.63

Discussion

All vital signs during anesthesia showed no significant difference at any time point and all were maintained within acceptable ranges. The results showed no major changes in any oxidative stress biomarker between baseline and any time point. Nevertheless, that could imply that 3 h of high oxygen exposure does not increase oxidative stress more than does low oxygen.

The 100% oxygen exposed mice died from pulmonary oxygen toxicity after continuous exposure for 3 to 4 d (11). Exposure time is an important factor that can lead to pulmonary toxicity (12). Our study demonstrated the effect of inspired oxygen for general inhalation anesthesia for 3 h in dogs, which might prove that high oxygen is safe under these conditions.

The d-ROM levels can be used to monitor oxidative stress because they serve as a marker of reactive oxygen species (ROS) in plasma; meanwhile, BAP levels can be used to monitor oxidative stress because they serve as a marker of reduced substances in plasma. High d-ROM levels imply increased production of ROS, while high BAP levels imply increased antioxidant capacity (13).

Both d-ROMs and BAP have been validated as reliable methods for evaluating oxidative stress in humans and animals (e.g., horses, rats, dogs) (13–17). After rats had been exposed to oxygen for 24 h, measurements of plasma d-ROM and BAP levels demonstrated that d-ROMs increased in groups with ≥ 40% oxygen, although there was no change in BAP (14). However, our data did not show a significant difference in d-ROM levels according to O_2 concentrations in dogs. The degree of oxidative stress affected the production of ROS and changes in d-ROM and BAP levels were seen after 3 d and 1 wk (16). Therefore, the changes only emerged after a considerable amount of time. Notably, the duration of administration of oxygen in the present study was short compared with that of Nagatomo et al (14).

The main antioxidant that counteracts ROS is SOD. The SOD level primarily increases according to the oxidative stress level. If oxidative stress increases, the SOD level should also increase, especially under 100% oxygen conditions. In this study, high levels of ROS in the alveoli were neutralized by overexpression of SOD. Notably, SOD can be classified into 2 types: i) intracellular SOD (i.e., MnSOD and Cu/ZnSOD located in the mitochondria and cytosol, respectively) and ii) extracellular SOD (i.e., Ec-SOD located in extracellular fluids and typically found in plasma) (18). When the production of ROS increases, Ec-SOD moves from the plasma to areas of inflammation, according to neutrophil and macrophage activity (18). Superoxide dismutase and other antioxidants fight against ROS. However, if there are more ROS than antioxidants, ROS will activate mitochondria and then cytochrome c is released in the cytosol, which leads to caspase activation and eventually, apoptosis. Thus, it was concluded that hyperoxia induces cell death (19). In 1991, O'Connell et al (20) reported that pulmonary mitochondria were significantly damaged after rats were exposed to 100% oxygen for 3 h. If high-concentration oxygen causes oxidative stress in the lung, plasma SOD should move to the lung, such that plasma SOD levels might suddenly decrease. Decreased plasma SOD stores will be replenished over time through homeostatic processes. We expected to observe this phenomenon in our 100% oxygen group, but no significant differences in plasma SOD were observed among groups. Intracellular ROS is produced by inflammatory cells, while plasma SOD is transferred to intracellular compartments by inflammatory cells to bolster the defense against ROS. In this situation, plasma SOD provides greater protection against injury from ROS. However, in our 40% and 60% oxygen groups, the concentration of SOD did not show a change from baseline at 3 h. This suggests that the presence of small amounts of intracellular ROS meant that plasma SOD was not required; therefore, the plasma SOD concentrations in both

Table III. Mean ± SEM plasma BAP concentrations (μmol/L) in the 3 oxygen concentration groups at 0, 3, 6, and 24 h after initiation of sevoflurane. P_{hours} are the comparisons among the 3 groups at each time point and $P_{100\%}$, $P_{60\%}$, and $P_{40\%}$ are the comparisons between baseline and each time point in the 100%, 60%, and 40% groups, respectively.

Hours	P_{hours}	100% group	$P_{100\%}$	60% group	$P_{60\%}$	40% group	$P_{40\%}$
0	0.93	3339.50 ± 555.30	—	3153.83 ± 345.11	—	2947.00 ± 247.59	—
3	0.74	3282.83 ± 470.78	0.87	2712.83 ± 437.46	0.42	3212.33 ± 636.58	0.63
6	0.91	2753.67 ± 356.74	0.34	2930.83 ± 310.79	0.63	3069.83 ± 522.96	0.87
24	0.98	2558.50 ± 260.08	0.42	2505.83 ± 128.41	0.15	2785.33 ± 356.67	0.52

Table IV. Mean ± SEM plasma SOD concentrations (U/mL) in the 3 oxygen concentration groups at 0, 3, 6, and 24 h after initiation of sevoflurane. P_{hours} are the comparisons among the 3 groups at each time point and $P_{100\%}$, $P_{60\%}$, and $P_{40\%}$ are the comparisons between baseline and each time point in the 100%, 60%, and 40% groups, respectively.

Hours	P_{hours}	100% group	$P_{100\%}$	60% group	$P_{60\%}$	40% group	$P_{40\%}$
0	0.37	1.72 ± 0.78	—	1.73 ± 1.03	—	1.16 ± 0.52	—
3	0.90	1.24 ± 0.77	0.20	1.68 ± 1.16	0.63	1.16 ± 0.34	0.87
6	0.93	1.93 ± 0.96	0.52	2.78 ± 1.97	0.34	2.07 ± 0.74	0.04*
24	0.96	2.72 ± 2.41	0.75	1.91 ± 0.89	0.42	1.72 ± 0.27	0.08

* Not statistically significant after Bonferroni correction.

Table V. Mean ± SEM plasma 8-OHdG concentrations (pg/mL) in the 3 oxygen concentration groups at 0, 3, 6, and 24 h after initiation of sevoflurane. P_{hours} are the comparisons among the 3 groups at each time point and $P_{100\%}$, $P_{60\%}$, and $P_{40\%}$ are the comparisons between baseline and each time point in the 100%, 60%, and 40% groups, respectively.

Hours	P_{hours}	100% group	$P_{100\%}$	60% group	$P_{60\%}$	40% group	$P_{40\%}$
0	0.93	392.44 ± 56.37	—	343.84 ± 28.30	—	352.41 ± 33.39	—
3	0.36	447.52 ± 57.53	0.42	342.23 ± 31.05	1.0	380.79 ± 53.01	0.63
6	0.68	385.55 ± 44.56	1.0	330.01 ± 21.53	0.63	360.79 ± 22.64	0.87
24	0.75	377.19 ± 51.08	1.0	376.85 ± 31.52	0.42	340.76 ± 18.65	0.75

groups remained constant. Thus far, there have been few reports of changes in plasma SOD concentrations due to oxygen exposure, but there have been many studies on SOD in tissues, such as the lung (21–23). By examining SOD levels in tissues that are directly injured, more detailed knowledge of the function of SOD upon exposure to high-concentration oxygen can be achieved.

8-OHdG is a primary product caused by the damaging effects of hydroxyl radicals on DNA. In recent years, this biomarker has been measured in studies of oxidative stress. Kumar et al (24) reported that plasma 8-OHdG increased over time in resuscitated premature infants receiving 21%, 40%, or 100% oxygen, but the levels did not differ among the oxygen groups. However, 8-OHdG levels were significantly higher at 4 wk than at 24 h. In our study, plasma 8-OHdG levels were not significantly different among the oxygen groups at any time point. This suggests that measuring plasma 8-OHdG may be useful for up to 4 wk after exposure to high oxygen. Jin et al (25) reported that there were no significant changes in 8-OHdG levels in rat lung tissues after 1 d of hyperoxia (90% oxygen) but there were significant changes after more than 2 d of hyperoxia exposure. This suggests that 3 h of hyperoxia exposure does not increase the level of oxidative stress. However, the previous study of plasma 8-OHdG was performed in humans, so further studies in dogs are needed (24).

Many studies have reported that exposure to high levels of oxygen causes increased oxidative stress in rats (20,26). Clinically significant pleural effusion, hyperpermeability of pulmonary capillaries, and pulmonary edema were observed in rats exposed to 100% oxygen for 48 h (26). However, Kumar (27) speculated that antioxidant enzyme activity may be exhibited differently in species; the activity of some antioxidant enzymes did not increase in oxygen-exposed rabbits, despite these being key enzymes in humans. In the present study, SOD levels were not significantly different between dogs exposed to high and low concentrations of oxygen.

Limitations of this study

This study investigated oxidative stress due to oxygen exposure for 3 h. However, as mentioned above, in several previous studies, oxidative stress was measured following 24-hour oxygen exposure (14,20). In addition, while our samples were collected 24 h after oxygen exposure, some researchers collected theirs between 36 and 48 h (14). Further studies should include longer oxygen exposure and/or sampling times.

In conclusion, d-ROM, BAP, SOD, or 8-OHdG levels do not change the oxidative stress level among oxygen groups at any time point. This suggests that compared to lower concentrations of oxygen,

100% oxygen may not alter the oxidative stress level and cause harm to dogs during general anesthesia with sevoflurane for at least 3 h.

Even in human medicine, it is controversial whether hyperoxia during inhalation anesthesia is considered good or bad. As mentioned above, high oxygen concentrations are regarded as toxic but may protect against hypoxia and prevent death in some cases. An important difference between human and veterinary medicine is that animals are generally anesthetized by technicians or general practitioners who lack extensive experience in anesthesia practice, whereas specialists perform anesthesia induction in human medicine. In addition, the monitoring equipment in veterinary medicine is generally of low quality and limited availability. To reduce inspired oxygen, measurements of inspired oxygen are needed. However, the equipment required to measure inspired oxygen is often unavailable, even in veterinary universities. High oxygen concentrations can increase oxidative stress; however, our study suggests that the production of oxidative stress is not enough to increase biomarker levels. High oxygen might be harmful but can be useful in certain situations.

To our knowledge, this study is the first to report on the use of high and low concentrations of oxygen for anesthesia in dogs and describe how 100% oxygen may not alter the oxidative stress level in dogs during general anesthesia with sevoflurane for at least 3 h, relative to lower concentrations.

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